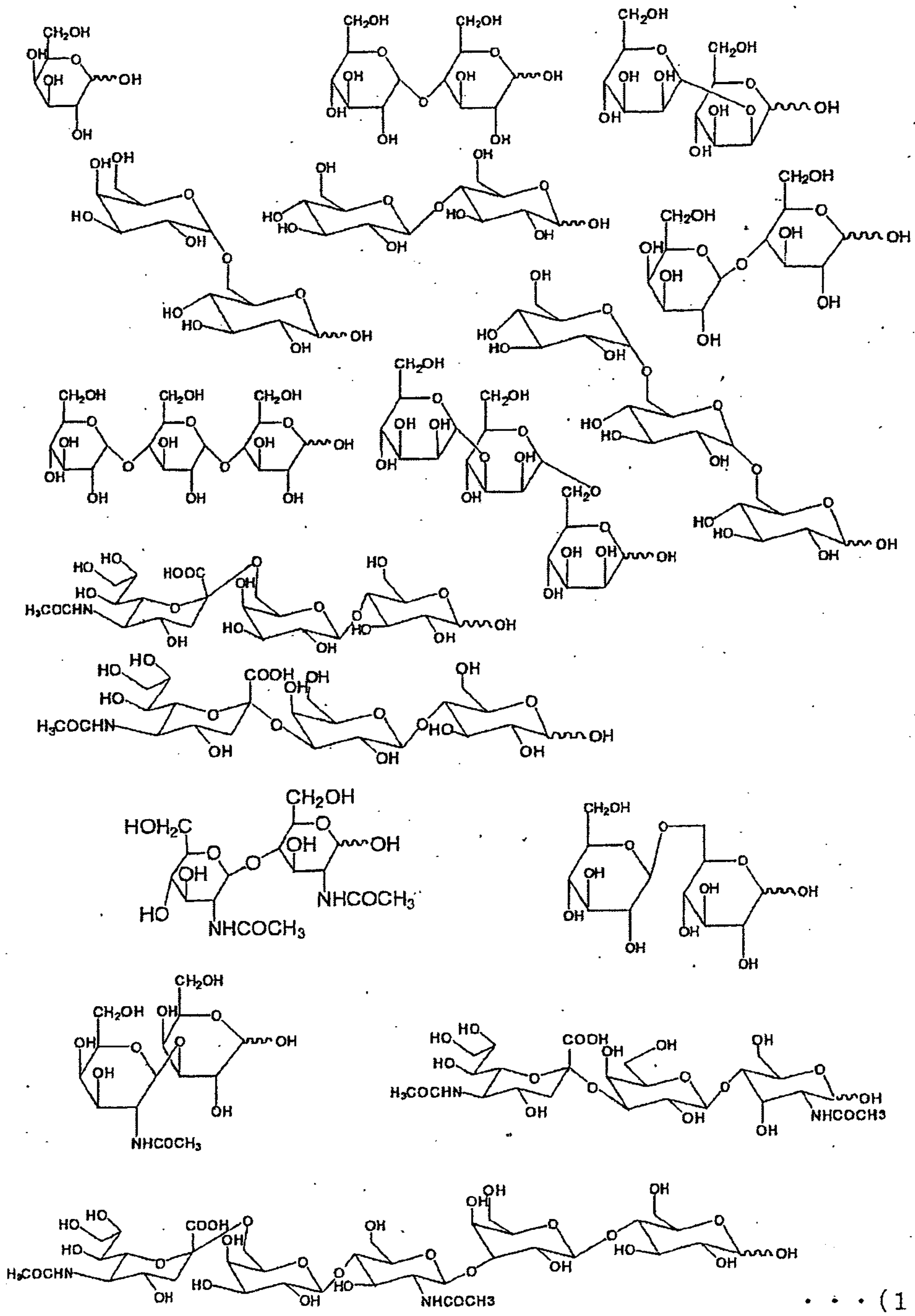
English Translation of Amendments under PCT Article 34 ②
filed on May 11, 2006



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10/590045
IAP12 Rec'd PCT/PTO 17 AUG 2006

English Translation of Amendments under PCT Article 34 ①
filed on December 16, 2005

hydrocarbon derivative chain. Note that in General Formula (9), n^1 and q are not particularly limited, provided that they are integers of not less than 0 but not more than 6. They may be different from one another, or may be the same integer.

The linker compound having the structure represented by General Formula (10) is a linker compound having one hydrocarbon derivative chain. Note that in General Formula (10), n^2 is not particularly limited, provided that it is an integer of not less than 1 but not more than 6.

The linker compound having the structure represented by General Formula (11) is a dimer of one hydrocarbon derivative chain. Note that in General Formula (11), n^1 is not particularly limited, provided that it is an integer of not less than 1 but not more than 6.

The "X" may have a structure of a multibranching moiety in which plural hydrocarbon derivative chains are bonded at an atom such as a carbon, nitrogen, or the like to form a branching structure, like in General Formula (7) or (8). In case where the "X" contains a plurality of hydrocarbon derivative chains, it is preferable that these hydrocarbon derivative chains have the same structure. However, these hydrocarbon derivative chains may have different structures, provided that they have an aromatic amino group at their ends.

As described above, the linker compound contained in the ligand conjugate according to the present invention contains a sulfur atom which can be bonded to the supporter for use in the protein analysis, and an amino group that can be bonded to a sugar molecule such as oligosaccharide chain, etc. Therefore, the linker compound is immobilized to the supporter for use in the protein analysis via a metal-sulfur bond such as Au-S bond. Thus, it is possible to firmly and easily bond the sugar molecule to the supporter via the linker compound.

under nitrogen atmosphere in the dark. The reaction solution was extracted with chloroform. The organic layer thus obtained was washed with water and with saturated saline, once each, and then dried with anhydrous magnesium sulfate. After the drying agent was filtered out, the filtrate was concentrated under reduced pressure, thereby to obtain a concentrated residue. The concentrated residue was purified via chromatography (10g, chloroform: acetone = 2:1), thereby obtaining azide compound (638mg, Yield: 90%) in the form of yellow liquid.

The azide compound (614mg, 2.01 mmol) was dissolved in 24ml of methanol. After 4.3ml of 1N NaOH was added therein at 0°C in the dark, the resultant solution was stirred at room temperature for 21 hours. The reaction solution thus obtained was concentrated under reduced pressure, thereby obtaining a concentrated residue. After chloroform was added to the concentrated residue, 1N HCl was added therein until pH 2 was obtained. Then, extraction with chloroform was carried out. The organic layer was washed once with saturated saline, and then dried with anhydrous magnesium sulfate. After the drying agent was filtered out, the filtrate was concentrated under reduced pressure, thereby obtaining Compound 16 (549mg, Yield: 90%) in the form of colorless liquid.

^{1}H-NMR (400MHz, $CDCl_3$) analysis of Compound 16 showed that δ6.19 (1H, bs, $CO_2\underline{H}$), 4.16 (2H, s, $OC\underline{H}_2CO_2H$), 3.75-3.64 (12H, m, $OC\underline{H}_2C\underline{H}_2O$), 3.68 (2H, m, $N_3CH_2C\underline{H}_2$), 3.41 (2H, t, J = 5.1 Hz, $N_3C\underline{H}_2$). Moreover, ESI-MS (negative) analysis of Compound 6 showed that m/z was 328.14 [$(M+Na)^+$]. This confirmed the structure of Compound <u>16</u>. In addition, Compound 16 was found to have a molecular mass of 277.13.

(2) Synthesis of Compound 17

Compound 16 (228mg, 0.823mmol) was dissolved in

anhydrous dichloromethane (4 ml). Then, at room temperature, HOBt (135mg, 0.987mmol), EDC·HCl (192mg, 0.987mmol), and then at 0°C Compound 11 (205mg, 0.987mmol) were added therein and then stirred for 20 hours in the dark. The reaction solution thus obtained was concentrated under reduced pressure thereby obtaining a concentrated residue. The concentrated residue was then extracted with chloroform. The organic layer thus obtained was washed with 10% citric acid, and with saturated sodium hydrogen carbonate aqueous solution, once each, and then dried with anhydrous magnesium sulfate acting as a drying agent. After the drying agent was filtered out, the filtrate was concentrated under reduced pressure, thereby obtaining a concentrated residue. The concentrated residue was purified via silica gel chromatography (80g, chloroform:methanol = 10:1), thereby obtaining Compound 17 (367mg, Yield: 95%) in the form of yellow oily material. ESI-MS (positive) analysis of Compound 17 showed that m/z was 490.24 [$(M+Na)^+$]. This confirmed the structure of Compound 17. In addition, Compound 17 was found to have a molecular mass of 467.24.

(3) Synthesis of Compound 18

Compound 17 (29mg, 0.062mmol) was dissolved in methanol (3ml). After 10% Pd/c (5.0mg) was added therein, the solution thus obtained was stirred for 9 hours under hydrogen atmosphere. After Pd/C was filtered out, the filtrate was concentrated under reduced pressure, thereby obtaining a concentrated residue. The concentrated residue was purified via silica gel chromatography (1.5g, chloroform: methanol = 7:1), thereby obtaining Compound 18 (22mg, Yield: 82%) in the form of yellow oily material. ESI-MS (positive) analysis of Compound 18 showed that m/z was 442.27 [$(M+H)^+$]. This confirmed the structure of Compound 18. In addition, Compound 18 was

1.31 (m 4H, $-COCH_2\underline{CH_2}CH_2\underline{CH_2}-$), 1.18 – 1.10 (m, 2H, $-COCH_2CH_2\underline{CH_2}CH_2-$). MALDI-TOF-MS analysis of Compound 22 showed that m/z was 878.39 [$(M+Na)^+$]. These confirmed the structure of Compound 22. In addition, Compound 22 was found to have a molecular mass of 855.35.

[Example 4: Synthesis of Ligand Conjugates (Compounds 26 and 27)]

In the present Example, ligand conjugates classified as seventh and eighth ligand conjugates explained in the embodiment were synthesized. That is, a ligand conjugate (Compound 26), which was a seventh ligand conjugate,_was synthesized as below, the ligand conjugate having a structure represented by General Formula (14), where n^1 was 3, X had a structure represented by General Formula (4), R' was a hydrogen (H), and R was glucose. Further, a ligand conjugate (Compound 26), which was an eighth ligand conjugate, was synthesized as below, the ligand conjugate having a structure represented by General Formula (14), where n^1 was 3, X had a structure represented by General Formula (4), R' was a hydrogen (H), and R was maltose.

Note that the analysis method, reagents, etc. are same as in Example 3.

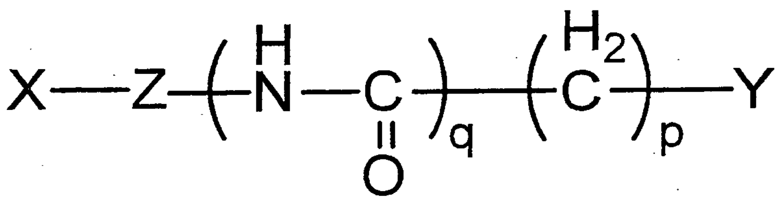
(1) Synthesis of Compound 24

The synthesis of Compound 24 is described below referring to Formula (32):

CLAIMS

1. (Amended) A ligand conjugate comprising a linker compound and a sugar chain bound to each other via an aromatic amino group,

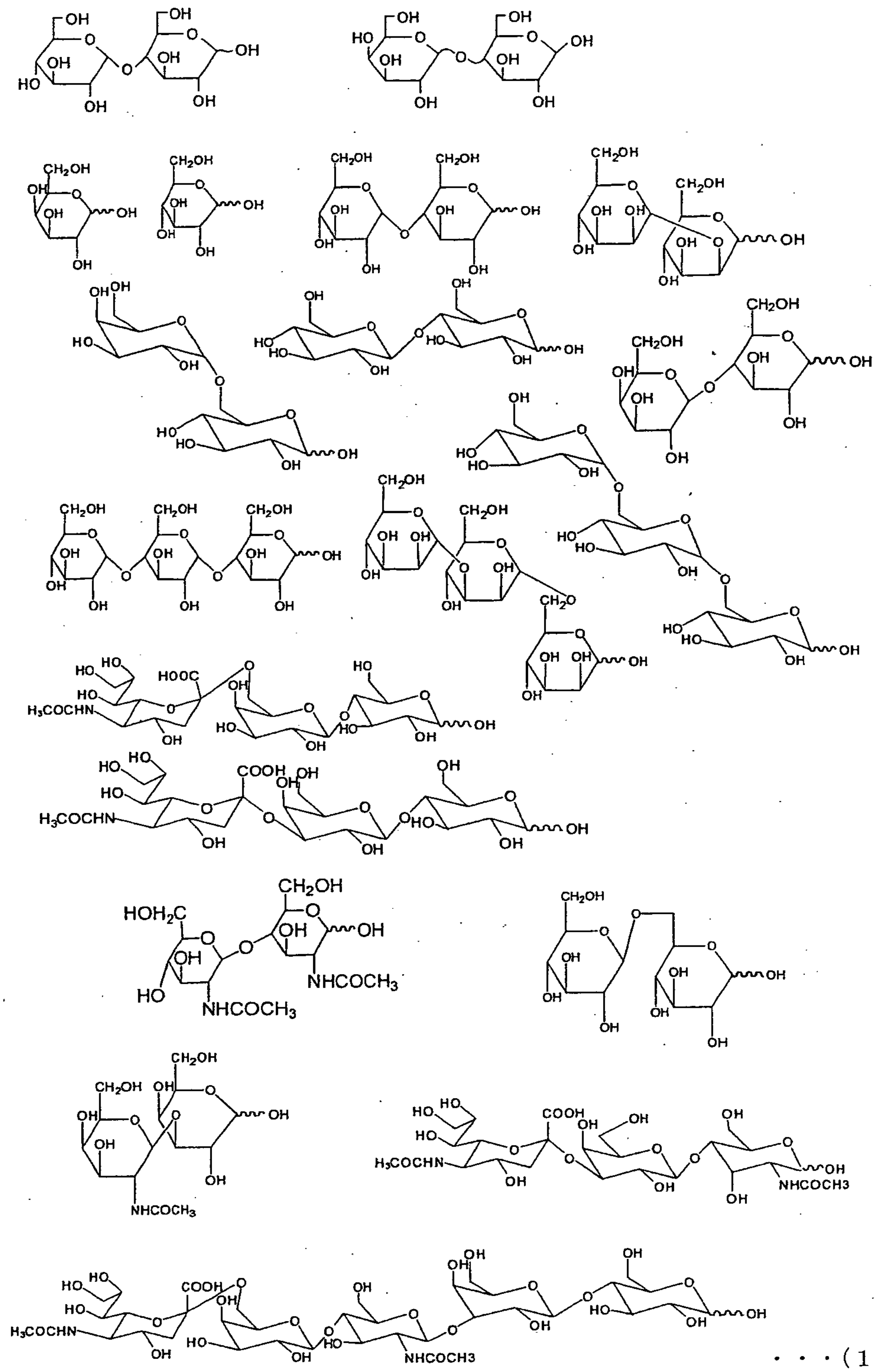
the linker compound having a structure represented by General Formula (1):



···(1)

where p and q are independently integers of not less than 0 but not more than 6, in which X is a structure comprising one, two, or three hydrocarbon derivative chains which have the aromatic amino group at an end and may have a carbon-nitrogen bond in a main chain, Y is a sulfur atom or a hydrocarbon structure containing a sulfur atom, and Z is a straight-chain structure comprising a carbon-carbon bond or carbon-oxygen bond,

the sugar chain being selected from Group (110):

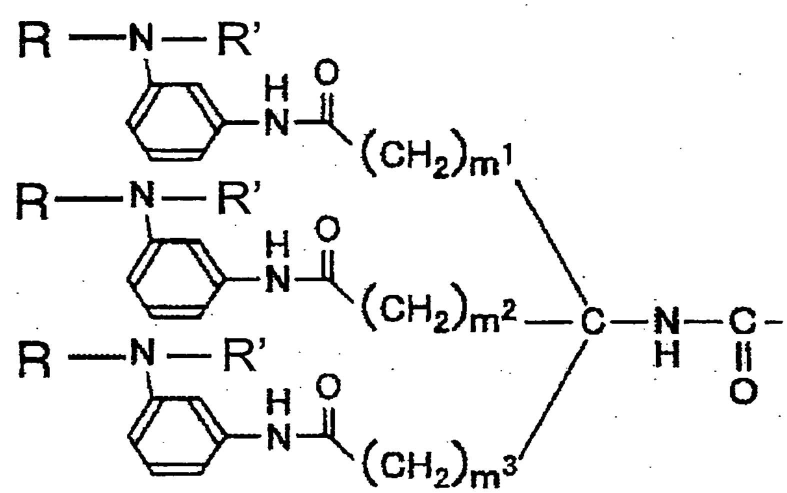


・・・(101)

2. The ligand conjugate as set forth in Claim 1, wherein Y is a hydrocarbon structure having a S-S bond or a SH group.

3. <u>(Amended)</u> The ligand conjugate as set forth in Claim 1 or 2, wherein:

X has a structure represented by General Formula (2):



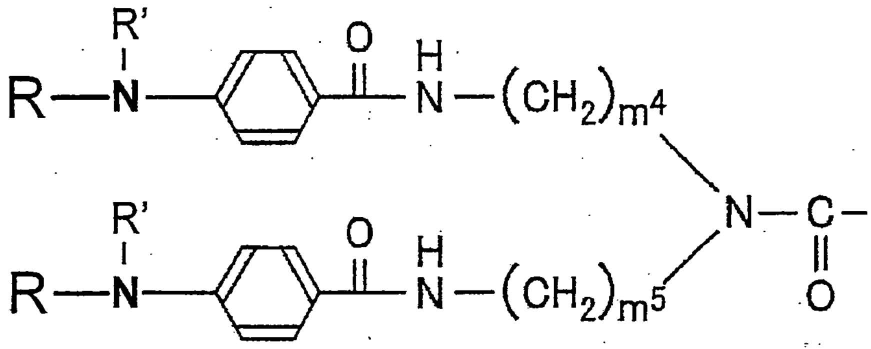
$\cdots$(2)

where m^1, m^2, and m^3 are independently integers of not less than 0 but not more than 6, and R' is a hydrogen (H) or R,

R being a compound derived from <u>the</u> sugar chain <u>selected from Group (101).</u>

4. <u>(Amended)</u> The ligand conjugate as set forth in Claim 1 or 2, wherein:

X has a structure represented by General Formula (3):



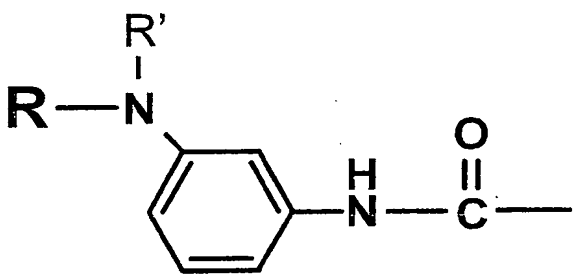
···(3)

where m^4 and m^5 are independently integers of not less than 0 but not more than 6, R' is a hydrogen (H) or R,

R being a compound derived from the sugar chain selected from Group (101).

5. (Amended) The ligand conjugate as set forth in 1 or 2, wherein:

X has a structure represented by General Formula (4):



···(4)

where R' is a hydrogen (H), or R,

R being a compound derived from the sugar chain selected from Group (101).

6. The ligand conjugate as set forth in Claim 1 or 2, wherein:

Z has a structure of Formula (5) or (6):

$$-\!\left(\overset{H_2}{C}\right)_{n^1}\!- \quad \cdots (5)$$

$$-\overset{H_2}{C}\!-\!\left(OCH_2CH_2\right)_{n^2}\!- \quad \cdots (6)$$

where n^1 and n^2 are independently integers of not less than 1 but not more than 6.

7. (Amended) The ligand conjugate as set forth in Claim 1 having:

______a structure represented by General Formula (107):

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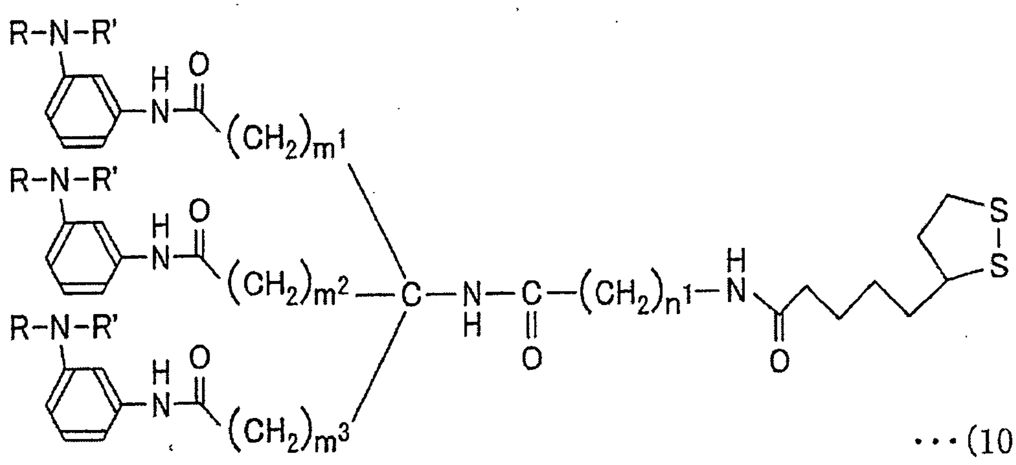
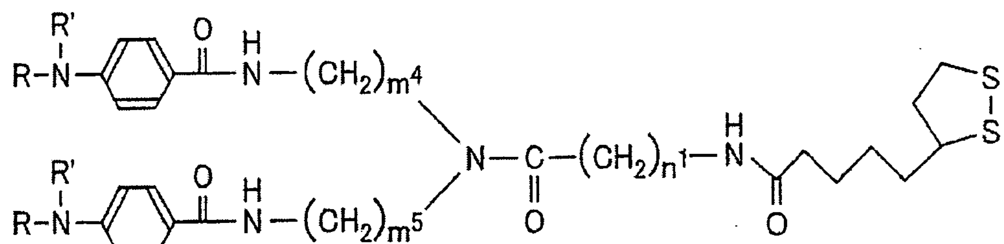


$\cdots$(107)

where m^1, m^2, and m^3 are independently integers of not less than 0 but not more than 6,_n^1 is an integer not less than 1 but

not more than 6, and R' is a hydrogen (H) or R;

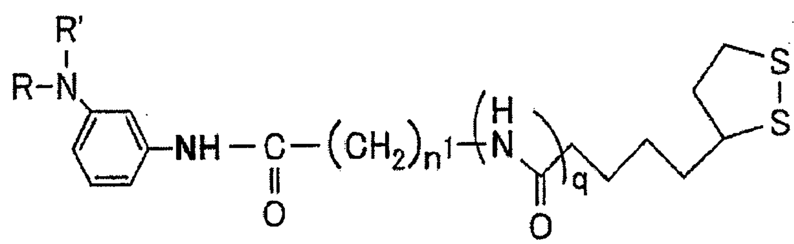
______a structure represented by General Formula (108):



$\cdots$ (108)

where m^4 and m^5 are independently integers of not less than 0 but not more than 6,_n^1 is an integer of not less than 1 but not more than 6, and R' is a hydrogen (H) or R;

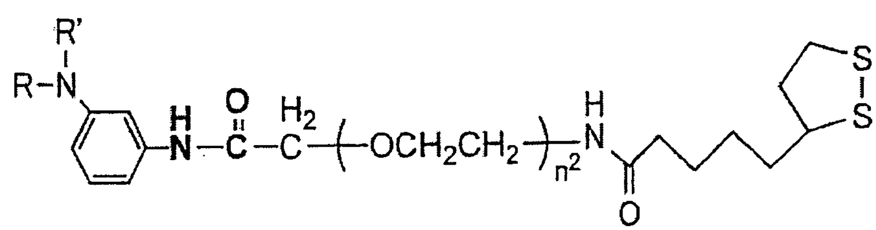
______a structure represented by General Formula (109):



$\cdots$ (109)

where n^1 and q are independently integers of not less than 0 but not more than 6, and R' is a hydrogen (H) or R;

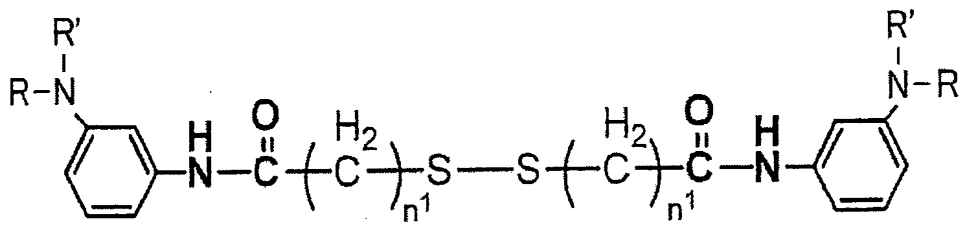
______a structure represented by General Formula (110):



$\cdots$ (110)

where n^2 is an integer of not less than 1 but not more than 6, and R' is a hydrogen (H) or R; or

______a structure represented by General Formula (111):



$\cdots(111)$

where n^1 is an integer of not less than 1 but not more than 6, and R' is a hydrogen (H) or R,

R being a compound derived from the sugar chain selected from Group (101).

8. (Amended) A ligand carrier in which the ligand conjugate as set forth in any one of Claims 1 to 7 is immobilized on a supporter having a metal on a surface thereof.

9. The ligand carrier as set forth in Claim 8 wherein the ligand carrier is used for protein analysis.

10. (Amended) A method for analyzing protein, comprising:

allowing the ligand conjugate as set forth in any one of Claims 1 to 7 to stand in contact with a supporter so as to prepare a ligand carrier in which the ligand conjugate is immobilized on the supporter;

analyzing intermolecular interaction after allowing the

ligand carrier to stand in contact with a protein solution; and

performing mass spectroscopy after the analysis of the intermolecular interaction, so as to identify a protein bound on the ligand carrier.